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The localization of key *Bacillus subtilis* penicillin binding proteins during cell growth is determined by substrate availability

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Supporting Information

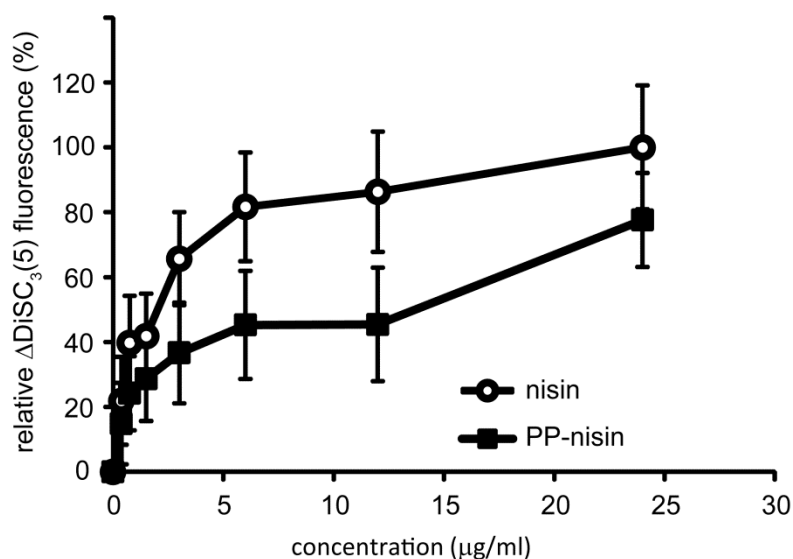


Figure S1. Lages et al.

Fig. S1. Fluorimetric measurement of the collapse of membrane potential by nisin and PP-nisin. The $\Delta\Psi$ -sensitive fluorescent dye $\text{DiSC}_3(5)$ accumulates on polarized membranes of glucose energized *B. subtilis* cells, which results in fluorescence quenching. Dissipation of $\Delta\Psi$ by nisin or PP-nisin is measured as release of the dye to the medium resulting in an increase in fluorescence. Various concentrations of nisin and PP-nisin were tested; values represent the mean and standard deviation from three different experiments that were performed in duplicate. Addition of nisin or PP-nisin at concentrations used to delocalize LipidII ($1.5 \mu\text{g ml}^{-1}$) resulted in a significant increase in $\text{DiSC}_3(5)$ fluorescence, indicative of a (partial) collapse of the membrane potential.

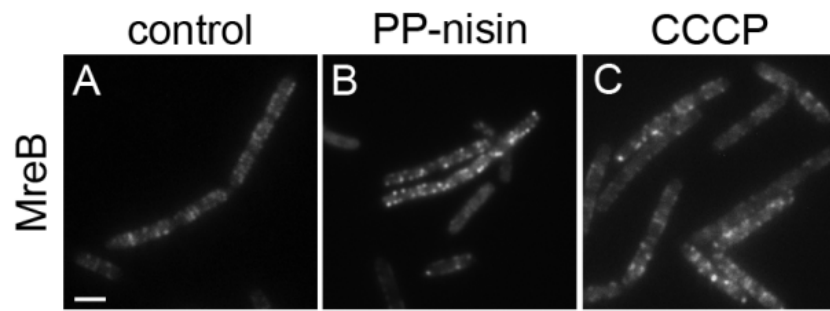


Figure S2. Lages et al.

Fig. S2. Delocalization of MreB by PP-nisin and CCCP. Localization of GFP-MreB in untreated cells (A) or after treatment with PP-nisin (B) or CCCP (C). Scale bar, same for all, 2 μ m.

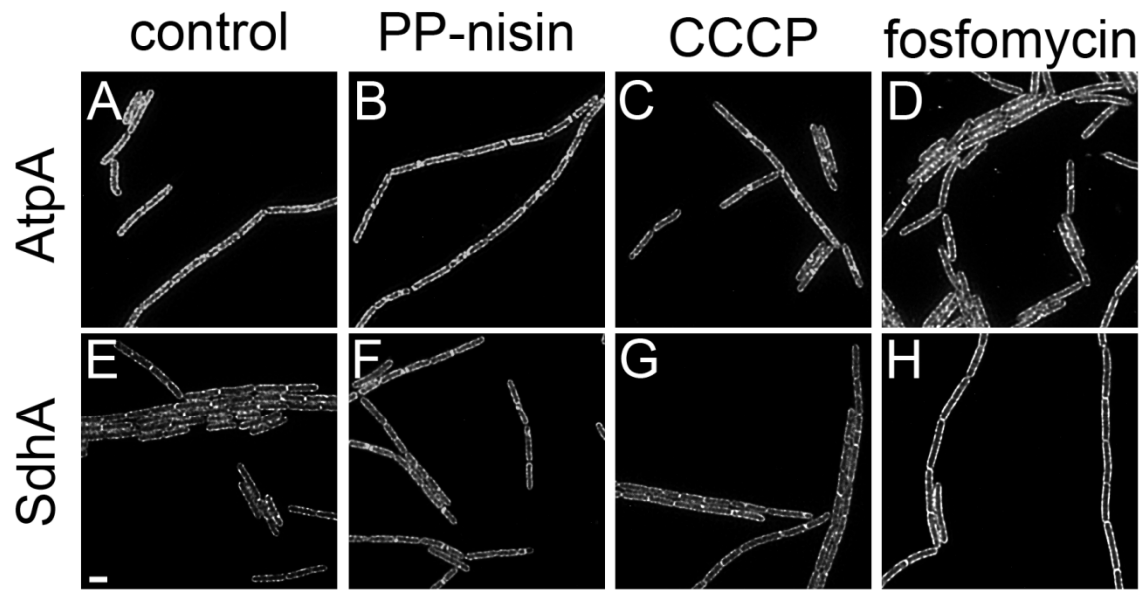
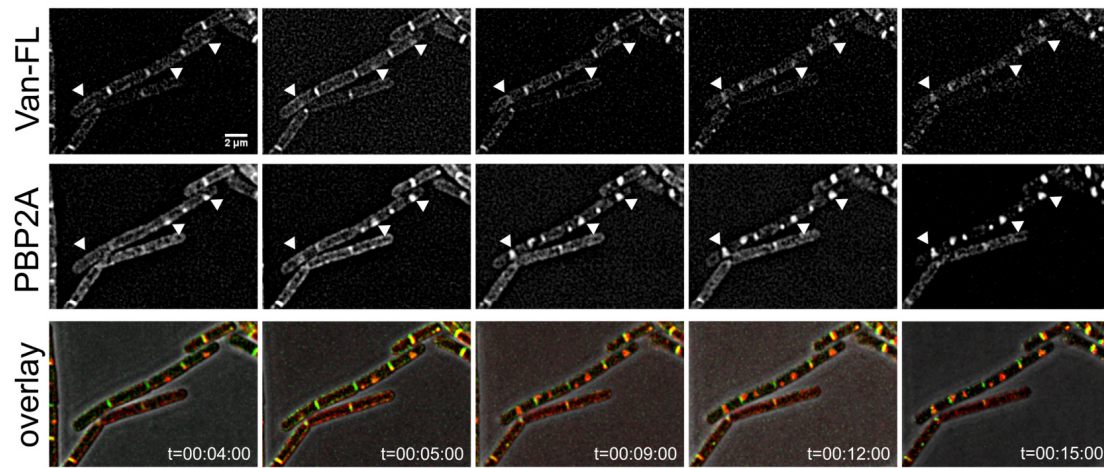


Fig. S3. Lages et al.

Fig. S3. Localization of AtpA-GFP and SdhA-GFP is not affected by PP-nisin or fosfomycin. Localization of AtpA-GFP (A-D) and SdhA-GFP (E-H) was performed in untreated cells (A,E) or after treatment with PP-nisin (B,F), CCCP (C,G), or fosfomycin (D,H). Scale bar, same for all, 2 μ m. To show clear membrane localization, background light was subtracted, and out-of-focus light was removed by two-dimensional-blind deconvolution as described (Johnson *et al.*, 2004).



Supplemental Figure 4. Lages et al.

Fig. S4. Time-lapse microscopy showing PG synthesis visualized by vancomycin-labelling (Van-FL, green in overlay) and localization of RFP-PBP2A (PBP2a, red in overlay) after treatment with PP-nisin. Time indicated in frames is minutes after the addition of PP-nisin. The images have been deconvolved. Scale bar 2 μm .

Movie S1. A montage of frames from the time-lapse microscopy images shown in Fig. S4.